

in the soil, since the conditions of the drain tile stimulate root development very materially. However, the root system of any tree or shrub is far in excess in length and area of what the layman imagines. The profuse growth of roots in water is also seen in cases where old wells become filled with root growth, but the pear tree root in question is one of the best examples which has ever come to our notice of root development in drain tile.

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THE NATURE AND FUNCTION OF THE PLANT OXIDASES

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(Continued from *February Torreya*)

PEROXIDASE

Besides the laccase and tyrosinase which we have been considering, there are other oxidizing enzymes which are not specific like the two mentioned. They act only in the presence of hydrogen peroxide, and therefore are called peroxidases. These enzymes have also been called "indirect oxidases" in distinction from those substances (Bach's oxygenases) which show their activity without the addition of peroxide as in the case of tyrosinase, etc. In 1903, Bach and Chodat¹⁵ discovered that by fractional precipitation of aqueous extracts of *Lactarius vellereus*, they were able to obtain two precipitates of very different properties. The fraction insoluble in 40 per cent. alcohol proved to be a direct oxidase, while the other fraction, soluble in 40 per cent. alcohol, but insoluble in 95 per cent. alcohol, had no direct oxidizing properties. With hydrogen peroxide and other peroxides, however, the second fraction showed strikingly peroxidase properties. Moreover, the peroxidase fraction, when allowed to act with the direct oxidase fraction, showed all the properties of

¹⁵ Bach and Chodat. Title of series is: Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zellen; V. Zerlegung der sogenannte Oxydasen in Oxygenasen und Peroxydasen. Ber. Chem. Gesell. 36: 606. 1903.

the original oxidizing substance as present in the plant. This research was the beginning of a series of notable contributions to our knowledge of the oxidizing enzymes. In another paper, these authors state that peroxidase is present in nearly every plant. They were able to prepare a pure peroxidase from the horseradish root, which exhibited great stability towards heat. In further comparative studies they showed that peroxidase greatly augments the power of the natural oxidases, especially that oxygenase from the same source as the peroxidase itself. All of these observations led Bach and Chodat to separate oxidases into two parts, the organic peroxide part, which they called "oxygenase" and the activator of oxygenase and other peroxides, to which alone they gave the name "peroxidase."

Kastle and Loevenhart¹⁶ in 1901 published a very important paper which has not always received due attention from the European chemists engaged in this work. These authors found that the substance bluing guaiacum directly is easily precipitated by alcohol and is destroyed by small amounts of hydrocyanic acid, hydroxyl amine and phenyl hydrazine. It seemed peculiar to them that these substances should be so harmful, but that sodium hyposulphite, silver nitrate and mercuric chloride, substances usually fatal to enzymes, should exert little effect on the constituent of the potato which blues guaiacum directly. In general, those substances which prevented the direct bluing of guaiacum tincture by the potato juice also prevented similar action upon guaiacum by the organic and inorganic peroxides with which they experimented. All of these experiments caused them to believe that this direct bluing was not due to enzymes at all, but to organic peroxides which were formed when the juice is exposed to the air, according to Engler's theories of auto-oxidation. Thus we see, the idea that oxidases are made up of an organic peroxide part activated by the enzyme peroxidase receives further confirmation from this work of Kastle and Loevenhart.

In a valuable paper by Kastle¹⁷ on "The Stability of the

¹⁶Kastle and Loevenhart. On the Nature of Certain Oxidizing Ferments. *Amer. Chem. Jour.* 26: 539. 1901.

¹⁷Kastle. On the Stability of the Oxidases, etc. *Bull.* 26, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv. Washington, 1906.

Oxidases," it appears that oxygenases of certain fungi are extremely resistant to the influence of both heat and long standing. In the case of the oxygenase from *Lepiota americana*, it was necessary to heat for several minutes to a temperature of about 85° in order to destroy the power of the extract to blue guaiacum directly. Still more striking is the case of the glycerin extracts of certain *Lactarius spp.*, which after standing from 1905 to 1909 were found to be still active towards both guaiacum and tyrosin. It is interesting to note that of the many species of the higher fungi which Kastle tested, only one, *Amanita verna*, did not show any response for the oxidases. This plant is so poisonous that it has been called the "destroying angel."

From all the experimental work of the different investigators it seems probable that peroxidase is an enzyme rather than a simple catalyzer. Little is really known of the nature of peroxidase. Bach¹⁸ has prepared a powerful peroxidase which gave no tests for proteins, nor did it contain iron or manganese. On the other hand, Van der Haar¹⁹ claims his *Hedera* oxidase was a glucoprotein. Resistance to heat seems to be a peculiarity of peroxidase. Heating to boiling is necessary to destroy peroxidase, while oxygenase is destroyed at a much lower temperature. Bach and Chodat noted this fact and also that upon standing after boiling, the peroxidase regained its activity. Woods²⁰ first discovered, this phenomenon while studying the peroxidase of the tobacco leaf, and concluded that in these cases we are dealing with a zymogen or a substance which regenerates the peroxidase upon standing. Aso²¹ also found that there were zymogens more stable towards heat than peroxidase itself, which slowly yielded more of the latter after the destruction of the initial supply. A second heating permanently destroys the peroxidase; the stronger the solution of the enzyme, the more resistant it is towards heat.

¹⁸ Bach. Zur Theorie der Oxydasenwirkung: I. Mangan und eisenfreie Oxydasen. Ber. Chem. Gesell. 43: 364. 1910.

¹⁹ Van der Haar. Untersuchungen in Pflanzenoxydasen: II. Die Hederaperoxydase, ein Glucoproteide. Ber. Chem. Gesell. 43: 1321. 1910.

²⁰ Woods. The Mosaic Disease of Tobacco. Report No. 18 [p. 17], U. S. Dept. Agric. 1902.

²¹ Aso. Which Compound Can Liberate Iodine from Potassium Iodide? Beihfte z. Botan. Centralblt. 15: 208. 1903.

The writer has also noted cases of the regeneration of the peroxidase after its apparent destruction by heat, especially in the case of the oxidase of the sweet-potato. Hasselbring and Alsberg²² have recently found that only in the presence of coagulable protein are the oxidases easily destroyed by heat.

With the exception of catalase there is probably no enzyme more common among plants and animals than peroxidase. There is hardly a plant or any part of its organs that does not blue tincture of guaiacum in the presence of hydrogen peroxide, thus proving the presence of peroxidases. The oxidases also play an important part in many industrial processes. The curing of tobacco, the production of the bouquet of wines, and the formation of commercial indigo from *Indigofera anil* in India, all seem to be somewhat dependent upon the oxidases. Green tea is produced when the freshly picked leaves are immediately spread on hot plates which, of course, destroys the oxidases, while the slow curing with consequent activity of the oxidases yields the black tea of commerce. The aroma of the vanilla-bean and the fragrance of the English meadow-sweet (*Ulmaria Ulmaria*) have also been attributed to oxidase action. Leptomin is really a peroxidase but Raciborski,²³ finding the indirect oxidase localized in the leptome (phloem) of plants, considered it a new enzyme, and one distinct from the direct oxidase. With guaiacum and hydrogen peroxide the strongest bluing is localized in the phloem through which the sieve-tubes pass, the latter acting as carriers of the food materials of the plant. This so-called leptomin is present in largest amount in the phloem of the latex plants. These illustrations will serve to show the distribution and importance of the oxidases in plants.

CATALASE

It has long been known that finely divided metals, blood, plant juices and fluids from the animal body cause the rapid decomposition of hydrogen peroxide. But this fact did not

²²Hasselbring and Alsberg. Studies upon Oxidases [an abstract]. Science II. 31: 637. 1910.

²³Raciborski. Ein Inhalts-körper des Leptoms. Ber. Botan. Gesell. 16: 52. 1898.

attract special attention at first because it was generally thought that the power to decompose hydrogen peroxide was a property common to all ferments (enzymes). However, beginning in 1888 with Bergengrün, different investigators discovered that the power to decompose hydrogen peroxide into oxygen and water could exist independently of the ordinary activities of such enzymes as the oxidases, diastase, emulsin, etc. Gottstein stated that the power of cells to break up hydrogen peroxide is due to their nucleic acid content and not to any enzyme, and furthermore, this power is shown after the death of the cell as well as during life. In 1901, Loew²⁴ found, in his studies on the enzymes of the tobacco leaf, that these leaves often caused a very active evolution of gas from hydrogen peroxide, but yielded none of the tests for oxidases, protein digesting enzymes, and other enzymes. This led him to study the matter more fully, with the result that by precipitation of the leaf extracts with ammonium sulphate and subsequent purification by alcohol precipitation, he obtained preparations that were extremely active in decomposing hydrogen peroxide, but which had no other property agreeing with the other classes of enzymes, such as the starch digesting action of diastase, etc. He named this substance "catalase" and considered that it was a new enzyme. Loew then made a more careful study of catalase and found that it apparently existed in two forms, α -catalase, which is insoluble in water, and the β -catalase, soluble in water. In a study of its distribution, Loew found that catalase is of practically universal occurrence in both plants and animals, a conclusion fully substantiated by the work of all later investigators. Recent observations made by Appleman²⁵ seem to show that catalase may be separated into a water-soluble and -insoluble portion as was previously claimed by Loew.

Euler²⁶ investigated the catalase of the fungus *Boletus scaber* in a painstaking manner. This catalase proved to be more sensitive to acids than animal preparations, but like them, there seemed to be some connection between the fat content of the

²⁴Loew. Catalase, a New Enzyme of General Occurrence. Report No. 68, U. S. Dept. Agric. 1901.

²⁵Appleman. Some Observations on Catalase. Bot. Gazette 50: 182. 1910.

²⁶Euler. Zur Kenntniss der Katalase. Hofmeister's Beitrage, 7: 1. 1908.

fungi and the amount of their catalase. Like the other investigators, he found that in dilute solutions and with a relative excess of the enzyme solution, the reaction followed the equation for reactions of the first order, thus tending to show that active oxygen was formed. In some cases he found that the physico-chemical constant k' equalled 0.0107 at 15° , this value for k' being identical with that found by Bredig and his collaborators for a colloidal platinum solution containing 0.006 gram of the metal per liter. The enzyme solution used by Euler in this determination contained 0.004 gram of enzyme preparation per liter. This enzyme was associated with globulin, but, taking the molecular weight as 1000, while that of platinum is 195, then $0.006/195$ N equals the concentration of platinum and $0.004/1000$ N equals the concentration of enzyme. This will give one an approximate idea of the tremendous catalytic activity of both of these substances. Not only do colloidal metal solutions and the vegetable catalases act in the same quantitative manner, but they also show the same sensitiveness to chemicals.

It seems likely that there is an antagonistic action between peroxidase and catalase. Shaffer²⁷ found that if uric acid were allowed to stand for several days with hydrogen peroxide solution, it was oxidized, but in the presence of catalase and hydrogen peroxide, there was no oxidation of the uric acid. This led Shaffer to believe that the spontaneous decomposition of the hydrogen peroxide results in the formation of traces of active oxygen, while that set free under the influence of catalase is wholly in the molecular (inactive) state. The main point of Shaffer's publication is that the oxygen set free by catalase is not in a nascent state and therefore catalase may have a certain protective power in the oxidation processes carried on by the cell. Herliztka²⁸ agreed with Shaffer that catalase has a protective action in the presence of peroxides or peroxidases. He also made quantitative studies on the oxidation of guaiacum by peroxidase and found a retarding action in the oxidation whenever catalase

²⁷ Shaffer. Some Observations on the Enzyme Catalase. *Am. Jour. Physiol.* **14**: 299. 1905.

²⁸ Herliztka. Ricerche sulla catalasi; Sull'antagonismo tra catalasi e perossidasi. *Rendic. Accad. Lincei. Atti. V.* **16**: 493. 1906.

was present. Bach showed that in a mixture of catalase and peroxidase the latter did not have an appreciable effect upon the action of the catalase. As we shall see in discussing the rôle of catalase in the cell, it is possible that it acts as a brake on the processes carried on by the oxidases.

In the catalytic decomposition of hydrogen peroxide into water and oxygen there has long been a controversy in regard to the nature of the oxygen evolved; that is, whether it is in the active state or in the inactive molecular condition. Now, in the case of catalase we know from the results of Shaffer and others, that no active oxygen is formed in the process, because guaiacum is not blued, and none of the reactions of nascent oxygen are shown; and furthermore, as Shaffer pointed out, if catalase produced active oxygen in the living cell, the protoplasm would probably be killed at once by this extremely active and destructive agent. How are we to harmonize those of the physico-chemical measurements with the results of Shaffer, Liebermann and others? From the physico-chemical data, the oxygen is in an atomic state, while from tests on the reaction mixture, it is apparently in a molecular state! We may say that the greater weight of evidence seems to favor the idea that the oxygen is in the inactive state and not capable of oxidizing *directly*.

In concluding this short discussion of catalase, we are forced to admit that our knowledge of this subject is very imperfect, and Cohnheim²⁹ voiced the thoughts of many investigators when he said: "It may well be that catalase is not an enzyme at all, but that the catalytic decomposition of hydrogen peroxide is a function of the large surfaces exposed by colloidal molecules, whether of organized matter or of metals in colloidal solution, the 'inorganic ferments' of Bredig."³⁰

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(To be continued)

²⁹ Cohnheim. Lecture at the New York University and Bellevue Hospital Medical College, New York City, December 10, 1909.

³⁰ Bredig. Die Anorganische Fermente, 1901.